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Case Report

Whole-genome analysis of SARS-CoV-2 samples indicate no tissue specific genetic adaptation of the virus in COVID-19 patients' upper and lower respiratory tract

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ABSTRACT

Sample panels of SARS-CoV-2 cases were retrospectively whole-genome sequenced. In three individuals, samples of upper and lower respiratory tract resulted in identical sequences suggesting virus stability including the spike protein cleavage site. In a fourth case, low-level intra-host genomic evolution and a unique 5-nucleotide deletion was observed.

1. Introduction

While SARS-CoV mainly affects the lower respiratory tract tissue, SARS-CoV-2 replicates effectively in the nasopharynx leading to a more efficient transmission of the virus (Wölfel et al., 2020). Irrespective of the severity of SARS-CoV-2 infection, the virus consistently replicates in the upper and lower respiratory epithelia (Weiss et al., 2020). Until now, sequence stability of SARS-CoV-2 in the upper and lower respiratory tract (URT and LRT, respectively) is insufficient explored. Therefore, sample panels comprising different clinical specimen of lethal SARS-CoV-2 cases were retrospectively investigated (Augsburg study (Schaller et al., 2020), Supplementary Table 1). After decease, postmortem analyses were performed between April 6 and May 13, 2020 ($n = 13$, Fig. 1A); samples were taken from different sites of URT and LRT and were tested for SARS-CoV-2 RNA using RT-qPCR (Supplementary Table 2). Selected samples were subsequently used for high-throughput sequencing (HTS) to compare whole-genome sequences, which originated from different specimens per case (URT vs. LTR, Fig. 1A). HTS was performed with samples when more than one sample per case was available and/or the RT-qPCR cycle threshold values were ≤25. Methods are described in the Supplementary Methods.

With this rationale, 13 whole-genome SARS-CoV-2 sequences were obtained. Four cases (AS-007, AS-011, AS-012, AS-015) were represented by two to four sequences obtained from different clinical samples. In two cases, all analyzed SARS-CoV-2 whole-genome sequences obtained from one individual were identical, in particular pharynx and/or trachea, bronchus, and pleura samples (cases marked in blue and red, Fig. 1B). These individuals (AS-011, AS-012, as well as AS-008 represented by only one sample) had been infected with the same virus variant of SARS-CoV-2 lineage B.1.5 (Rambaut et al., 2020). Sequences marked in yellow (AS-015) differ from the sequences of the blue/red/grey cluster and could be assigned to SARS-CoV-2 lineage B.1.1. The case with the most divergent sequences of the study, in relation to the reference, is represented by the green-marked samples, assigned to SARS-CoV-2 lineage B.40 (case AS-007). The sequences show the closest relationship to sequences reported from Jamaica and France (Supplementary Figure 1), and go back to an

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original virus variant exhibiting an S-614D type that was circulating early in the pandemic until it was replaced by the more infectious S-614G type (Korber et al., 2020). The sampling date of the green-marked case AS-007 (April 2020) matches with the main circulation period of this virus type. Furthermore, these S-614D type sequences found in pharyngeal and pleura samples showed a 5-nucleotide (nt) deletion in ORF8 (Fig. 1B), which leads to a truncation of 15 amino acids when CTG can be used as an alternative start codon, and introduces a frameshift (Fig. 1C). If no alternative start codon can be used, the protein would be completely lost. Such a 5-nt deletion of ORF8 was not described before, i.e., it is not existing in any SARS-CoV-2 sequence submitted to NCBI so far (until March 12, 2021). In addition to the deletion, we detected one transition (position 2492, Fig. 1B) in the SARS-CoV-2 sequence of the pharynx sample compared with the pleura sample. Single nucleotide variants (SNVs) were not detected in any of the analyzed samples except sample 4032 (pharynx, green-marked case AS-007). Here, we detected two SNVs, namely T9817C (frequency 24.4%) and C12775T (frequency 12.7%; 0.53 strand bias for both variants), that were both not found in the corresponding pleura sample. Importantly, none of the sequences showed any changes or SNVs in the SARS-CoV-2 spike protein cleavage site.

Intra-host variability during the course of COVID-19 was investigated and compartmentalized virus replication was observed in former SARS-CoV-2 studies (Jary et al., 2020; Rueca et al., 2020). However, neither significant differences regarding nucleotide diversity and SNVs nor a compartment-specific pattern were detected between samples from different respiratory compartments (Rueca et al., 2020). Also in our study, not investigating the evolutionary process during the disease but rather the result of potential intra-host evolution in postmortem samples, we could not find any indication that a specific adaptation to different compartments of the respiratory tract is necessary for efficient virus replication and severe disease. Despite the apparent stability, some single nucleotide changes could be seen between samples from the upper and the lower respiratory tract in one case. Our findings indicate some low-level intra-host genomic evolution of the virus of this individual case but depending on the tissue in which the virus replicated (pharynx) and maybe the virus type. Avanzato and colleagues reported a prolonged virus shedding by an immunocompromised individual with cancer including a clear intra-host evolution of the SARS-CoV-2 virus during the prolonged infection (Avanzato et al., 2020). The patient of the green-marked case (AS-007) underwent a hospitalization period of seven days until decease. The onset of typical COVID-19 symptoms was six days before admission (Supplementary Table 3). No special peculiarities during the course of disease possibly connected to COVID-19 could be specified (Supplementary Table 4). The soon decease might be due to the comorbidities, i.e., a malign lung tumor in progressed stage (poorly differentiated adenocarcinoma stage 4) besides atrial fibrillation, and aortic valve stenosis (Supplementary Table 5). However, we cannot fully exclude a longer persistence of the infection before clinical admission. With respect to the unique ORF8 deletion detected in this patient, different deletions within the SARS-CoV-2 ORF8 have been described before (Flower et al., 2021; Pereira, 2020; Su et al., 2020). The accessory gene encoded by ORF8 is suspected to have an important role in immune response of the host (Flower et al., 2021; Weiss et al., 2020).

Despite the small patient cohort, our results do not indicate a general necessity of tissue specific adaptation but rather an accidental occurrence of SNVs; they also suggest stability of the spike protein cleavage site within different compartments of patient's respiratory tract. However, sequences of one individual showed a unique deletion of ORF8 and additionally some sequence differences between the URT and LRT sequences. This might be a patient-induced effect due to poor health condition of the individual. In addition, it can only be speculated whether a longer-term replication of the virus could occur as in the case of other described ORF8 deletions. Overall, based on the present data with a limited sample size, this provides a hint and needs to be proven in further cohort studies. Furthermore, the study underlines the importance of whole-genome sequencing to detect variants and to reveal intra-host evolution of the virus.

Author contribution

BM, JS and MB conceived the study. BM, TS, KH and RC performed the autopsies, BM, OS, KH collected clinical data. SD, EK performed the primary swab analyses by PCR, CW sequenced genomes and analyzed data. CW, BM, DH, MB wrote the paper. All authors conducted the study, collected data, read and corrected the manuscript and approved the final version.

Ethics statement

This study was approved by the local institutional review board (2020-18), and written informed consent was obtained from next of kin.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.diagmicrobio.2021.115520.

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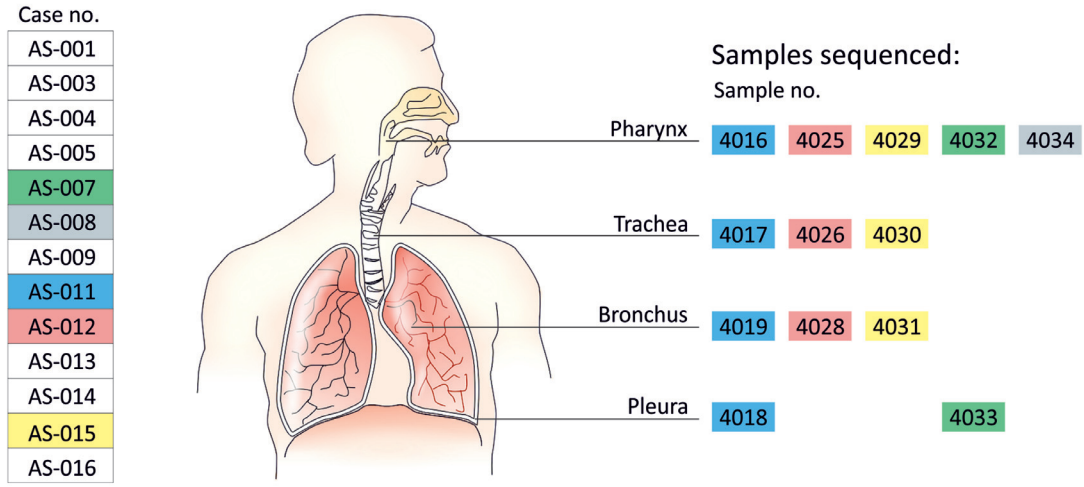
A. Overview of relations between analyzed cases (n=13) and derived samples (of 5 cases)



B. Nucleotide exchanges detected in SARS-CoV-2 genome sequences

Nucleotide in reference:			T	A	C	T	G	G	G	C	C	C	A	G	G	ACCAA	G	GGG	C	G
			Position of nucleotide exchange according to the reference (*first nucleotide each)																	
Case	Sample	Mean sequence coverage	253	2492	2570	3049	8191	10267	11095	14420	14817	14860	20280	23415	26156	27955*	28715	28893*	29750	29759
AS-007	4032	16.838	**C**	**G**	**T**	**C**	**T**	G	**T**	C	**T**	**T**	A	**A**	**T**	-----	G	GGG	C	G
	4033	205	**C**	A	**T**	**C**	**T**	G	**T**	C	**T**	**T**	A	**A**	**T**	-----	G	GGG	C	G
AS-008	4034	44.793	T	A	C	T	G	**T**	G	**T**	C	C	**G**	G	G	ACCAA	**T**	GGG	C	G
AS-011	4016	3.741	T	A	C	T	G	**T**	G	**T**	C	C	**G**	G	G	ACCAA	**T**	GGG	C	G
	4017	522	T	A	C	T	G	**T**	G	**T**	C	C	**G**	G	G	ACCAA	**T**	GGG	C	G
	4018	576	T	A	C	T	G	**T**	G	**T**	C	C	**G**	G	G	ACCAA	**T**	GGG	C	G
	4019	2.533	T	A	C	T	G	**T**	G	**T**	C	C	**G**	G	G	ACCAA	**T**	GGG	C	G
AS-012	4025	543	T	A	C	T	G	**T**	G	**T**	C	C	**G**	G	G	ACCAA	**T**	GGG	C	G
	4026	3501	T	A	C	T	G	**T**	G	**T**	C	C	**G**	G	G	ACCAA	**T**	GGG	C	G
	4028	14.322	T	A	C	T	G	**T**	G	**T**	C	C	**G**	G	G	ACCAA	**T**	GGG	C	G
AS-015	4029	11.972	T	A	C	T	G	G	G	**T**	C	C	A	G	G	ACCAA	G	**AAC**	**T**	**T**
	4030	9.511	T	A	C	T	G	G	G	**T**	C	C	A	G	G	ACCAA	G	**AAC**	**T**	**T**
	4031	5.151	T	A	C	T	G	G	G	**T**	C	C	A	G	G	ACCAA	G	**AAC**	**T**	**T**

C. ORF8 - frame shift detected in AS-007 (green-marked)

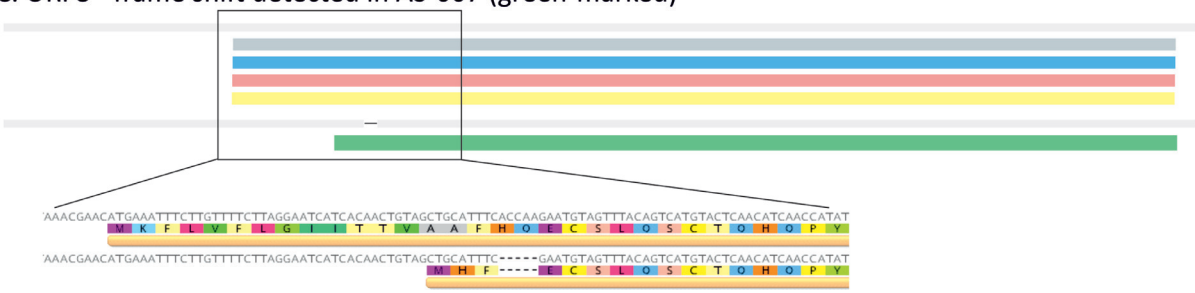


Fig. 1. Overview of sampling, diagnostic methods used and results. (A) Cases subjected to autopsy; RT-qPCR was performed with several samples of 13 COVID-19 deceased (results see Supplementary Table 2). SARS-CoV-2 whole-genome sequencing was performed for different samples of five marked cases. Respiratory tract was taken from pixabay (https://pixabay.com/). (B) Documentation of nucleotide exchanges in SARS-CoV-2 genomes found in five different individuals. Numbers above represent the nucleotide position of the exchange according to the reference SARS-CoV-2 isolate 2019_nCoV Muc IMB1 (first German case; accession number LR824570). Exchanges are marked in the color of the case. The nucleotide in position 23415 represents the S-614 type (nucleotide G stands for S-614G; nucleotide A stands for S-614D). (C) The 5-nucleotide deletion in position 26955 results in a frame shift in ORF8 (lower green-marked ORF8) in contrast to the ORF8 of the other cases with typical length. Light grey lines symbolize nucleotide sequences. The extended insert shows the nucleotide deletion with translation into amino acids downstream the beginning of ORF8